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Award Number: W81XWH-09-1-0134

TITLE: SPECT Imaging to Evaluate Post Traumatic Stress Disorder

PRINCIPAL INVESTIGATOR: John Seibyl, M.D.

CONTRACTING ORGANIZATION: Institute for Neurodegenerative Disorders
New Haven, CT 06510

REPORT DATE: February 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
February 2011	Annual	20 Jan 2010 – 19 Jan 2011

4. TITLE AND SUBTITLE

SPECT Imaging to Evaluate Post Traumatic Stress Disorder

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-09-1-0134

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

John Seibyl, M.D.

E-Mail: jseibyl@indd.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Institute for Neurodegenerative Disorders
New Haven, CT 06510

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Post traumatic stress disorder (PTSD) is a complex clinical disorder resulting from exposure to intense, life-threatening events resulting in persistent re-experiencing of the trauma, avoidance of stimuli associated with the trauma, dissociation, and heightened arousal which severely impact social and occupational functioning. Recent work has underscored morphological and functional brain alterations in PTSD patients using brain imaging with MRI, SPECT and PET imaging. Despite this encouraging preliminary work, there exists only a limited understanding of the pathophysiological changes which may subserve symptoms of PTSD. Preclinical studies now suggest that inflammatory changes may be implicated in neuronal loss in models of PTSD. Microglia represent a key inflammatory cell mediator within the CNS. Upon activation, these cells densely express an 18 kDa translocator protein (TSPO) receptors on their cell surface. Hence, it is possible to develop a radiotracer which targets TSPO as a marker for neuroinflammation. We have performed preliminary work with the TSPO imaging agent 123-I CLINDE with a goal of this proposal is to establish and validate an imaging biomarker for neuroinflammation in PTSD subjects that could both enhance our understanding of the pathophysiology and identify new therapeutic strategies for PTSD.

15. SUBJECT TERMS
Post traumatic stress disorder Brain imaging

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	14	19b. TELEPHONE NUMBER *(include area code)*

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Introduction

Activated microglia have been proposed to play a major role in the pathogenesis of several central nervous system conditions including post-traumatic stress disorder (PTSD). Microglia are a cell type that represents over 10% of the cells in the brain and become “activated” in response to various stimuli. This activation is thought to control areas of inflammation in the brain. In certain neurological diseases, this activation is thought to contribute to progression of neuronal damage. When microglia become activated they express peripheral benzodiazepine receptors (PBR) or binding sites on their mitochondrial membrane. PBRs are functionally and structurally distinct from central benzodiazepine receptors associated with gamma-aminiobutric acid (GABA)-regulated chloride channels. PBRs are found in abundance in peripheral organs and hematologic cells, but are present at only very low levels in the normal central nervous system (Banati, 2002).

A number of ligands with high in vitro affinity to PBR have been investigated for PET or SPECT imaging. To date, 11-C PK-11195 has been considered the best agent, in spite of its limitations (Kropholler, 2007; Turkheimer, 2007; Schuitemaker, 2007; Imaizumi, 2007). 123-I PK-11195 was also evaluated in humans but its low brain penetration makes it unfavorable for further evaluation (Shinotoh, 2006; Dumont, 1999; Gildersleeve, 1996). More recently, 18-F and 11-C labeled DAA1106 analogs were shown to be promising for imaging in nonhuman primates, but to date no report has been published concerning their use in humans (Boutin [65]. PET imaging using 11-C PK11195, a PBR agent, has demonstrated increased uptake in patients with several neurodegenerative conditions. Although 11-C PK11195 has provided critical proof of concept that in vivo imaging may be used to monitor neuroinflammation, there are several limitations to this ligand. Quantitation of 11-C PK11195 is difficult and use of 11-C labeled ligands are not practical in large clinical studies because of the short half-life (20 min).

In this proposal we planned to develop a PBR-binding radiotracer labeled with 123-I (T1/2 13.1 h) to enhance the clinical utility of imaging of neuroinflammatory targets. Unfortunately, 123-I CLINDE, the radiotracer initially proposed had limited signal:noise properties in other,human trials. We have subsequently identified a better agent for interrogating PBR in PTSD subjects, 18-F PBR111, a PET compound with more favorable human preliminary data than 123-I CLINDE. PBR111 appears to bind selectively to the PBR, also referred to as TSPO (the 18kDa translocator protein). An increase PBR111 binding to PBR is a potential marker of microglial activation in the CNS. The increase in PBR111 binding may be an indicator of the transition of microglia from a resting to an activated state, reflecting inflammation and its clinical sequelae relevant to PTSD symptomatology.

Body

This is a Phase 1, single-center, open-label, non-randomized, clinical study in PTSD and HCs to evaluate the kinetics, clearance and cerebral distribution of [18F]PBR-111 as outlined in the study protocol on file with the DOD. Investigational new drug approval has been granted by the FDA to study this agent (IND # 107,622). The

underlying goal of this study is to assess [18F]PBR-111 PET imaging as a tool to detect microglial activation in the brain of PTSD research participants and similarly aged healthy subjects. All study procedures will be conducted at the Institute for Neurodegenerative Disorders (IND) in New Haven, CT.

Approximately 8 subjects with post-traumatic stress disorder (PTSD) and 6 similarly aged healthy controls will be recruited to participate in this study. Previous studies with novel radiotracers in healthy and disease cohorts suggests there sample sizes are sufficient to detect 20% differences for outcome measures with good test-retest reproducibility. Healthy controls will be screened to ensure that there is no evidence of significant neurological or psychiatric disturbance.

The study population will consist of male or female subjects of any ethnic group with the features of PTSD and HC subjects. The study investigator will confirm (or reject) the diagnosis of PTSD based on the DSM-IV criteria after clinical and neuro-psychiatric examination. Only subjects fulfilling all inclusion criteria and none of the exclusion criteria for PTSD and healthy controls will be included into the study.
To determine the efficacy of [18F]PBR-111 PET scans in subjects with PTSD and HCs on the basis of neocortical tracer binding pattern, the PET scans will be assessed by a nuclear physician experienced in the field of neuro-imaging. PET scan findings will be classified either as abnormal (i.e., significant neocortical uptake in predefined regions) or as normal (i.e. no significant neocortical uptake in predefined regions). The nuclear physician will be unaware of the clinical diagnosis.

In support of this study, we have assessed [18F]-PBR111 as a potential marker for microglial activation in association with neuronal damage in previous human and non-human primate PET studies to determine the suitability of the radiotracer for interrogating multiple central nervous system conditions including PTSD. These studies are briefly described below.

Key Research Accomplishments

We decided to consider additional TSPO agents with better properties prior to exposing PTSD subjects and controls to imaging studies with a poor tracer. In this vein, outside the context and funding of the present proposal, we initiated human studies with three additional TSPO radioligands with the aim of quickly deciding upon a more optimal radioligand for the PTSD work. This report summarizes the progress of this work, even though we realize it is not being supported or part of the research initiatives funded under this grant. In addition, we have contacted the grant administrator to indicate we would wish to request a no cost extension of the work to facilitate the best scientific study possible under the current award. Summarizing this, objective progress in support of the initiation of the PTSD trial includes:

1. We have established collaborations with colleagues in the Yale Department of Psychiatry (Drs. Steven Southwick and John Krystal) for assistance in the referral and evaluation of PTSD research participants.

2. Obtained an IND for 18F-PBR111 for human use.

3. Finalized technical aspects of radiochemistry, PET acquisition, radiometabolite analysis, and image processing and signal quantification in support of the current study.

4. Completed all DOD human research approval requirements.

The issues and work delineated in point 3 above are described in the subsequent portion of this report.

Reportable Outcomes

Review of additional clinical imaging data from studies in controls and selected neuropsychiatric disorders

The following reports preliminary work done with 18F PBR111, unfunded by, but in support of the current study.

A. [18]-F PBR111 PET Baboon studies

PET investigations were conducted involving the bolus injection of [18]-F PBR111 in three ovariectomized female baboons (Papio anubis). Briefly, [18]-F PBR111 was prepared by halogen exchange of the appropriate precursor with [18F]fluoride in the presence of potassium carbonate and kryptofix-222 followed by HPLC isolation. Images were acquired after administration of 4.60 to 5.85 mCi of [18]-F PBR111 injected as an intravenous bolus in three female ovariectomized baboons (weight 12.10 – 18.65 kg). The animals were studied under isoflurane anesthesia after ketamine and rubinol induction. Serial dynamic brain PET acquisitions were acquired for up to 3-h post injection. PET studies were analyzed to evaluate the maximal uptake of radioactivity and washout, pattern of metabolite formation in blood, and effect of displacing agent PK11195 on specific binding in different brain regions.

Safety data
Following the induction of anesthesia through the end of the study heart rate, respiratory rate, blood pressure, and temperature were monitored every fifteen minutes. Data from the anesthesia record were entered into a database and to assess alterations in vital signs following injection of [18]-F PBR111. The animal tolerated the injection of [18]-F PBR111 and scan procedures without significant adverse effects. There were no systematic effects noted on vital signs following [18]-F PBR111 injection.Vital sign data from a representative study is indicated in Fig A1 below.

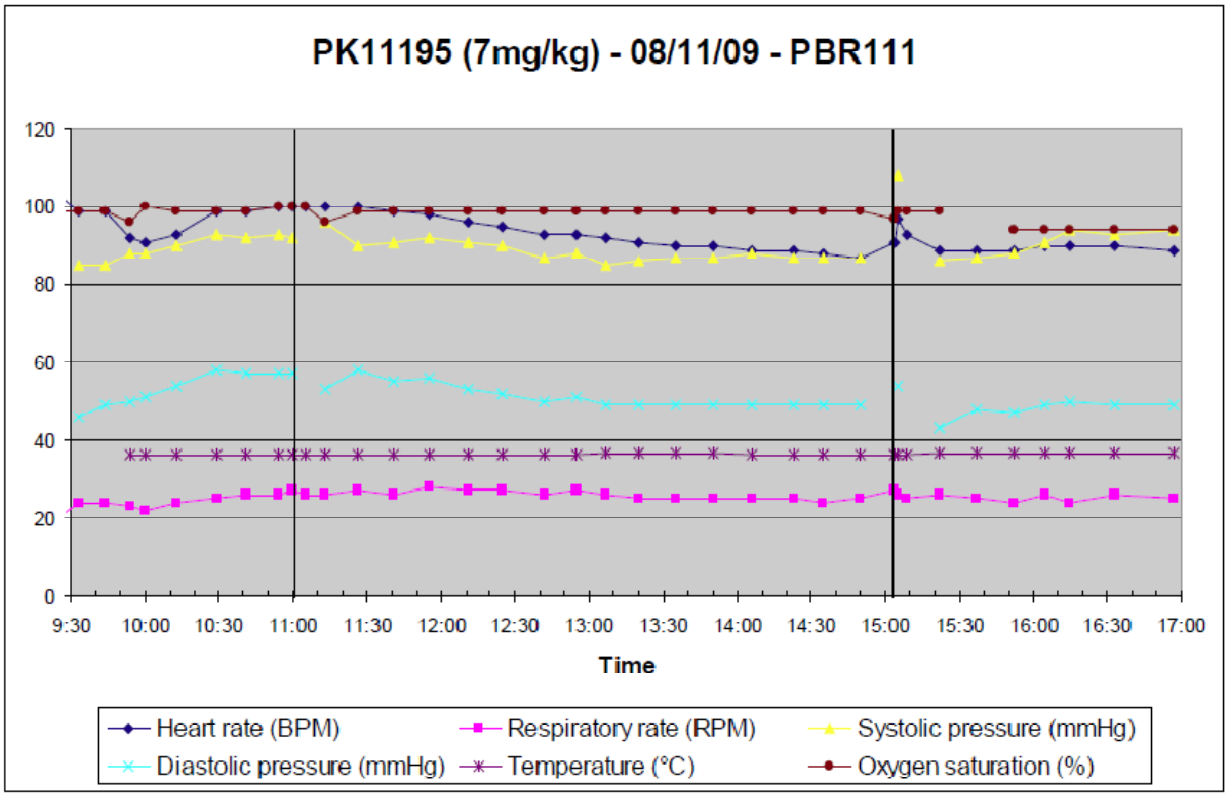

Figure A1. Panel indicates vital signs in a female baboon following intravenous injection of[18F]-PBR111(5.04 mCi) and PK11195 (7 mg/kg). The two vertical lines on the graph indicate the time points at which the radiotracer and displacing agent were injected, respectively.

B. [18F]-PBR111 PET Human studies

A total of 11 human subjects (8 healthy volunteers, 3 Alzheimer's subjects)have been studied with [18]-F PBR111 in the context of separately funded and approved investigations to validate and optimize the imaging outcome measure for the current proposal. These studies are performed under IND # 107,622 at the Institute for Neurodegenerative Disorders, New Haven, CT.

Subject ID	Mind #	Gender	Diagnosis	Dose mCi	MBq
PBR111-02	292-07378	M	HC	4.72	174.64
PBR111-01	292-07412	M	HC	4.41	163.17
PBR111-03	292-07428	F	HC	3.95	146.15
PBR111-05	292-07506	M	HC	5.00	185.00
PBR111-08	292-07607	M	HC	3.11	115.07
PBR111-09	292-07621	F	HC	4.97	183.89
PBR111-11	292-07622	F	HC	3.55	131.35
PBR111-12	292-07635	F	HC	5.01	185.37
PBR111-08	292-07658	M	HC	5.01	185.37
PBR111-09	292-07668	F	HC	3.00	111.00
PBR111-05	292-07506	M	HC	4.69	173.53

Subject ID	Mind #	Gender	Diagnosis	Dose	MBq
PBR111-04	292-07474	M	AD	4.59	169.83
PBR111-06	292-07533	M	AD	3.49	129.13
PBR111-13	292-07683	M	AD	4.92	182.04

Table A1. Human PBR111 PET subjects studied to date include 8 healthy volunteers (HC) and 3 Alzheimer's (AD) subjects.

Following bolus injection of [18]-F PBR111, serial dynamic PET brain acquisitions were obtained for three hours. Arterial and venous sampling was obtained for determining the metabolite corrected brain input function. Images were reconstructed with a standard iterative algorithm with scatter, randoms, and attenuation correction applied. Each serial image volume was spatially normalized and co-registerred with a T1 weighted MRI and a standardized volume of interest brain template based on a the AAL template was checked for accuracy on the MRI then transfered to the PET image for extraction of standard upatke values (SUV). These regional brain SUVS were then modeled with classic two tissue (2T), one tissue (1T), and Logan plot kinetic analyses to determine Vt. In addition, a pixelwise analysis (2T) was performed on the image volumes using modeled constraints determined in the VOI-level analysis.

Findings: All subjects tolerated the PET procedures without subjective or objective changes following radiotracer injection. Specifically, there were no changes in vital signs, EKG measures, serum chemistries, hematological indices, or somatic complaints relative to baseline assessments.

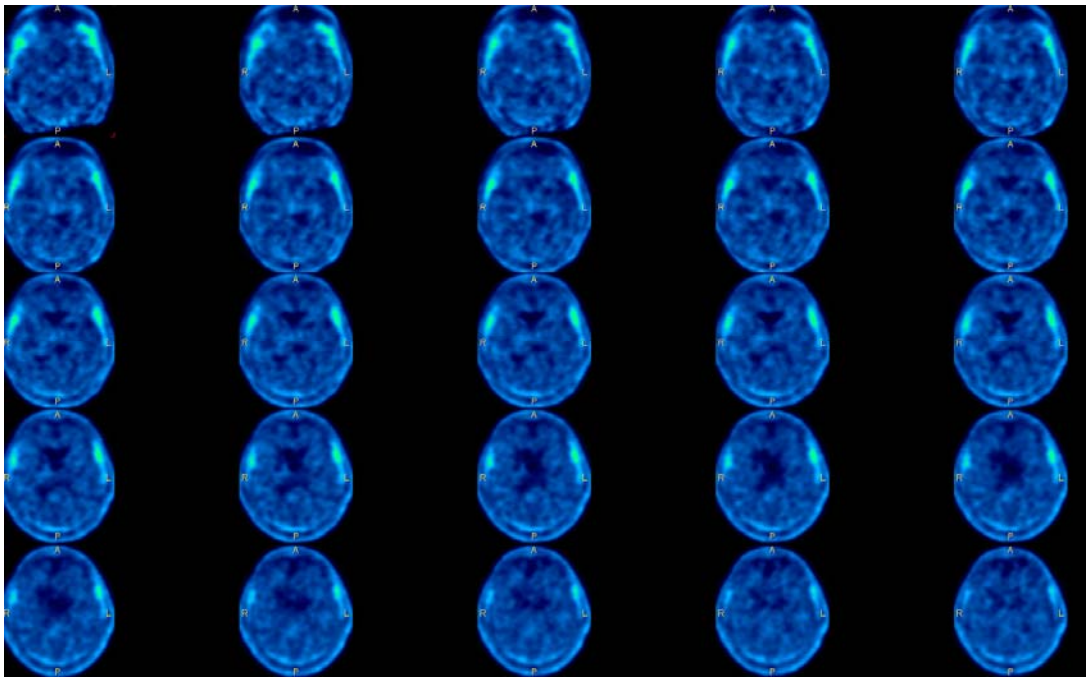

Figure A2: Representative PET axial images obtained from a healthy human volunteer receiving a bolus injection of [18]F PBR111.

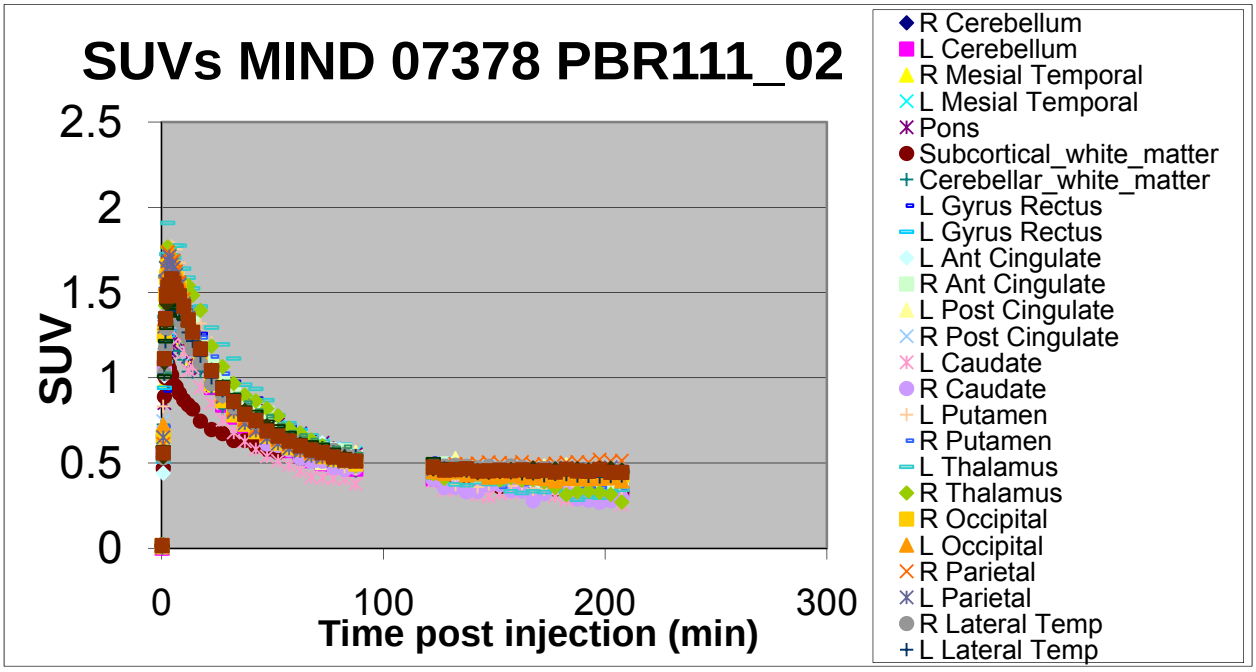

Figure A3: Standard uptake value (SUV) time-activity curve for multiple brain regions in a healthy human subject receiving a bolus injection of [18]F PBR111. The gap in the curve indicates a rest period outside the camera when no images were acquired.

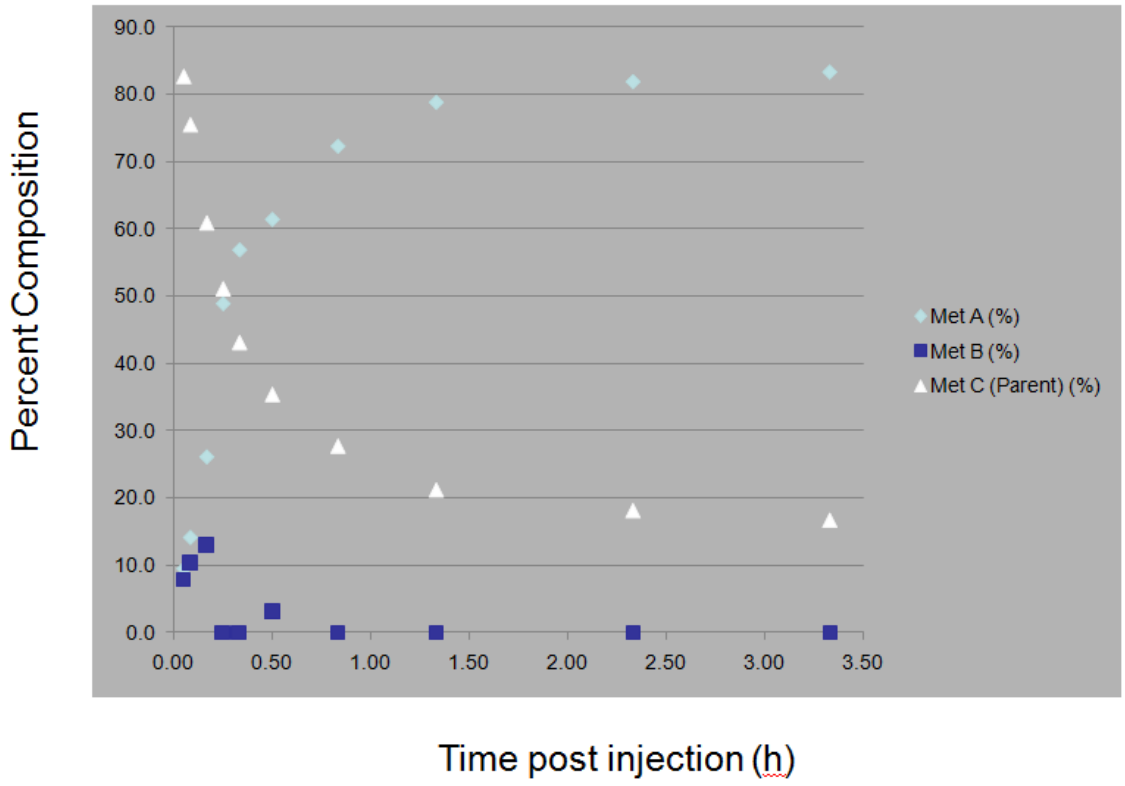

Fig A4: Plasma analysis in healthy human volunteer indicating metabolism of [18]F PBR111 into two metabolites with about 22% of parent compound present at one h post injection.

Analysis of blood data demonstrated the following:

1) Venous plasma analysis demonstrates parent compound is quickly metabolized with residual parent reflecting 10-20% of activity at 90 minutes and some variability between subjects.

2) PBR111 is primarily metabolized to two extractable metabolites- one of which is predominant (Metabolite A= 50-90%, Metabolite B = 0-30%). No evidence of confounding lipophilic metabolite.

3) There are no apparent differences in subjects with regard to radiotracer metabolism.

Analysis of PET brain data suggests:

1) Good initial brain penetrance and uptake with relatively fast washout.

2)Two tissue compartment models well-characterize the data, including good fits over the first 90 minutes post injection.

3) Delayed appearance of skull uptake affects quantitation when later time points are used, raising Vt- due to spillage into adjacent neocortical regions.

In summary, human validation studies of [18]F PBR111 demonstrate favorable metabolism and brain kinetics for kinetic modeling of TSPO receptor density (Vt or BPnd). There were no safety issues identified in any of the eleven subjects.

Conclusion

This report describes our scientific progress with our back-up TSPO PET agent 18F-PBR111 acquired in parallel projects evaluating neurodegenerative disease and suggests that we have adequate signal:noise properties to proceed with the approved PTSD study. All DOD and FDA regulatory requirements have been completed and we are now actively recruiting.

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Appendix

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